Growth and Metabolism of the Green Alga, *Chlorella Pyrenoidosa,* in Simulated Microgravity

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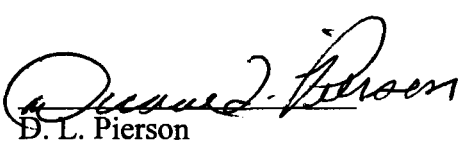

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Attachment 2

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Final Report

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Abstract

The effect of microgravity on living organisms during space flight has been a topic of interest for some time, and a substantial body of knowledge on the subject has accumulated. Despite this, comparatively little information is available regarding the influence of microgravity on algae, even though it has been suggested for long duration flight or occupancy in space that plant growth systems, including both higher plants and algae, are likely to be necessary for bioregenerative life support systems.

High-Aspect-Ratio Rotating-Wall Vessel or HARV bioreactors developed at Johnson Space Center provide a laboratory-based approach to investigating the effects of microgravity on cellular reactions. In this study, the HARV bioreactor was used to examine the influence of simulated microgravity on the growth and metabolism of the green alga, *Chlorella pyrenoidosa.*

After the first 2 days of culture, cell numbers increased more slowly in simulated microgravity than in the HARV gravity control; after 7 days, growth in simulated microgravity was just over half (58%) that of the gravity control and at 14 days it was less than half (42%). Chlorophyll and protein were also followed as indices of cell competence and function; as with growth, after 2-3 days, protein and chlorophyll levels were reduced in modeled microgravity compared to gravity controls.

Photosynthesis is a sensitive biochemical index of the fitness of photosynthetic organisms; thus, CO_2-dependent O_2 evolution was tested as a measure of photosynthetic capacity of cells grown in simulated microgravity. When data were expressed with respect to cell number, modeled microgravity appeared to have little effect on CO_2 fixation. Thus, even though the overall growth rate was lower for cells cultured in microgravity, the photosynthetic capacity of the cells appears to be unaffected.

Cells grown in simulated microgravity formed loose clumps or aggregates within about 2 days of culture, with aggregation increasing over time. Presently, the basis for, or significance of, the cell aggregation is unknown.

The results from this study suggest that cell growth and morphological characteristics of green algae may be altered by culture in simulated microgravity. The data obtained to date should provide a solid basis for additional experimentation regarding the influence of modeled microgravity on cell morphology, physiological activity, protein production and possibly gene expression in algal and plant cell systems. The final aim of the study is to provide useful information to elucidate the underlying mechanism for the biological effects of microgravity on cells.

INTRODUCTION AND BODY OF REPORT

Microgravity

The effect of microgravity on living organisms during space flight has been a topic of interest for some time, and a substantial body of knowledge on the subject has accumulated. Despite this, comparatively little information is available regarding the influence of microgravity on algae, even though it has been suggested that for long duration flight or occupancy in space, plant growth systems, including both higher plants and algae, are likely to be necessary for bioregenerative life support systems [13, 14]. Moreover, it has been stated that "an elucidation of the range and mechanisms of the biological effects of microgravity is one of the urgent fundamental tasks in space biology" [7].

The term "microgravity" is generally accepted as a condition in which the absolute sum of all mass-dependant accelerations does not exceed a certain small noise level, typically 10^{-5} g to 10^{-4}g. Cell cultures exposed to microgravity are influenced by at least three relevant factors: a) three dimensional cell assembly, b) low shear and turbulence, and c) co-spatial arrangement of different cell types and substrates [5].

Generation of Simulated Microgravity

A major advance for microgravity studies in cell culture has been the development of Rotating Wall Vessel or RWV bioreactors at NASA-Johnson Space Center [11]. The RWV bioreactors produce an environmental condition variously called "simulated or modeled microgravity" in which the gravitational vectors are randomized over the surface of the cells, resulting in an over-all-time-averaged gravitational vector of about 10^{-2} *g* [1]. This reduction in gravity creates a sustained low-shear environment for cell growth and is intended to model in the laboratory some effects of weightlessness or microgravity on cells (5,6).

A particularly useful form of RWV bioreactor is the High-Aspect-Ratio Rotating-Wall Vessel or HARV bioreactor. Figure 1 below illustrates how the HARV bioreactors may be oriented to grow cells under conditions of "simulated microgravity" (Fig. 1A) or normal gravity (1 x *g*) (Fig. 1B). When the unit is completely filled with liquid, gas bubbles cannot cause turbulence and a HARV, with its axis of rotation perpendicular to gravity, simulates microgravity by nullifying the downward gravity vector. A second HARV may be placed in a horizontal position. In this case, the axis of rotation is parallel

to the gravity vector and the gravity vector is no longer nullified, thus serving as a "gravity control".

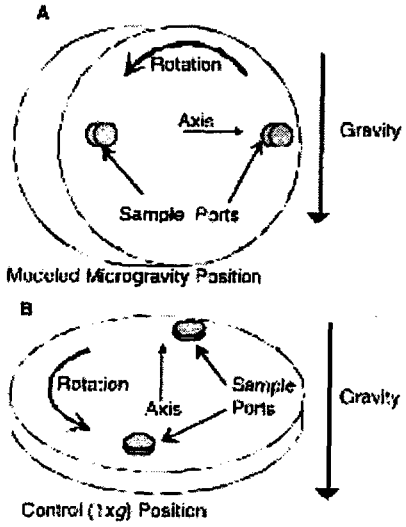

Figure 1. High-Aspect-Ratio Rotating-Wall Vessel Bioreactor (HARV). A HARV bioreactor in the modeled or simulated microgravity orientation (A) and in the normal gravity "control" position (B) is shown. Figure taken from Nickerson, et al. [8].

Previous Microgravity Studies in Algae

Over the last few years, rotating bioreactors have been used to study the effects of simulated microgravity on mammalian cells (see [12] for a review) and in bacteria [2-4]. By contrast, little work on the growth of algal and plant cells in modeled microgravity has been carried out using HARV or other RWV bioreactors.

The green alga, *Chlorella pyrenoidosa* has been used in a limited number of experiments in both space and clinostat studies. In these cases, *Chlorella* cells have been cultured in semiliquid and solid aseptic media, both under light and dark conditions. Under these conditions, an increase in biomass, reproduction and viability of *Chlorella* was observed when cells were grown in microgravity on space vehicles. The microgravity conditions not only affected growth but also structural and biochemical indices, suggesting that weightlessness may have diverse and important influences on growth and vital functions of physiologically active *Chlorella* [11].

A typical feature of *Chlorella* vegetative cells is the large cup-shaped chloroplast that occupies most of the cell's volume. Under autotrophic conditions, the thylakoids are joined in bundles and form the granae in separate chloroplast areas. The pyrenoid is perforated with one to three thylakoids and surrounded by amylogenic coating. When grown in darkness on an organic medium, the granae are absent and bundles of

thylakoids often bend [9]. Changes in the ultrastructure of *Chlorella* cells were seen in space experiments of different duration [10]. In short duration, 4.5 day, studies with *Chlorella* strain LARG-1 cultivated on a semiliquid medium, there was a decrease in the relative volume of thylakoids and starch grains in chloroplasts, and the cytoplasmic membrane had more complex folds. In 10.5 – 18 day flights, there were alterations in cytokinesis and dilation of the intrathylakoid membrane space. In addition, with the decrease in the thylakoid volume there was a simultaneous decrease in chlorophyll *a* and *b* content. There was an increase in condensed chromatin, which accumulated along the nuclear periphery, as well as an increase in cell vacuolization and number of mitochondria per cell. Finally, a doubling of specific amylases compared to ground control was seen [9, 10].

Specific Aims of the Study

The goal of this study was to examine the effect of microgravity on growth, morphological and biochemical characteristics of a green alga, in this case *Chlorella pyrenoidosa.* The specific aims of the research were the following:

1. To examine the effect of simulated microgravity on growth of *Chlorella* cells.
2. To study the effect of simulated microgravity on morphology of *Chlorella* cells.
3. To examine the effect of simulated microgravity on the production of chlorophyll and protein by *Chlorella* cells.
4. To study the effect of simulated microgravity on photosynthetic capacity of *Chlorella* cells.
5. To examine the effect of simulated microgravity on the protein expression of *Chlorella* cells.
6. To examine the effect of simulated microgravity on the gene expression of the *Chlorella* cells.

Results

Results from simulated microgravity studies with *Chlorella pyrenoidosa* are illustrated in Figures 2-12, as well as Table 2, below. Cultures were grown in either simulated microgravity or HARV gravity control conditions (see Figure 1 above). In addition, a culture grown in a non-rotating flask was used as an alternative gravity control.

Figure 2 shows growth data as determined by cell number. Cell numbers in simulated microgravity were similar to those in the HARV gravity control for the first 2 days. After that time, cells grew more slowly in simulated microgravity than in the HARV gravity control; by day 7 the growth in simulated microgravity was approximately half (58%) that in the HARV gravity control and by day 14 day it was less than half (42%). Interestingly, growth was substantially greater in the stationary flask gravity control (see Figure 2) than either the HARV gravity control or in simulated microgravity. Chlorophyll and proteins levels were also followed as indices of growth and cell viability; after 2-3

days of culture, both chlorophyll and protein were lower in cultures grown in simulated microgravity than in gravity controls (Figures 3 and 4).

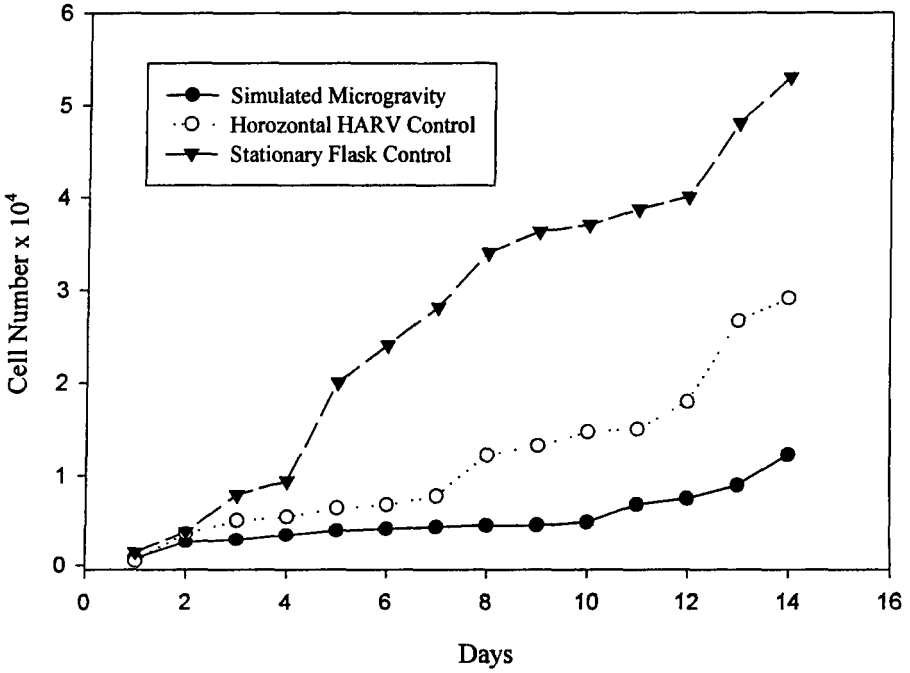

Figure 2. Effect of Simulated Microgravity on Growth of *Chlorella pyrenoidosa* Cells.

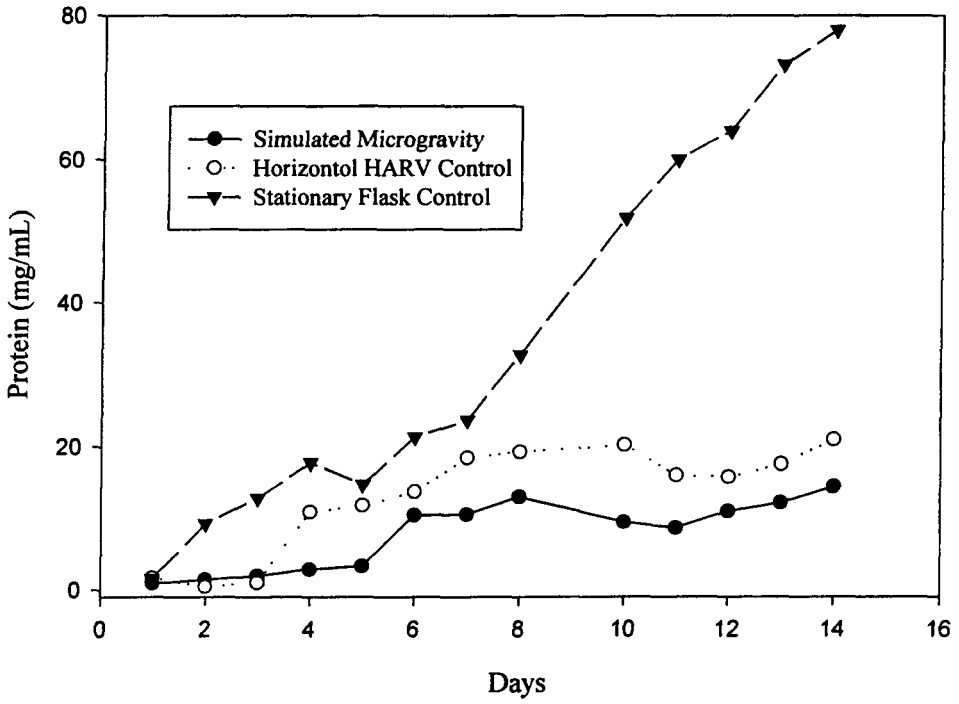

Figure 3. Effect of Simulated Microgravity on Protein Production in *Chlorella pyrenoidosa* Cells.

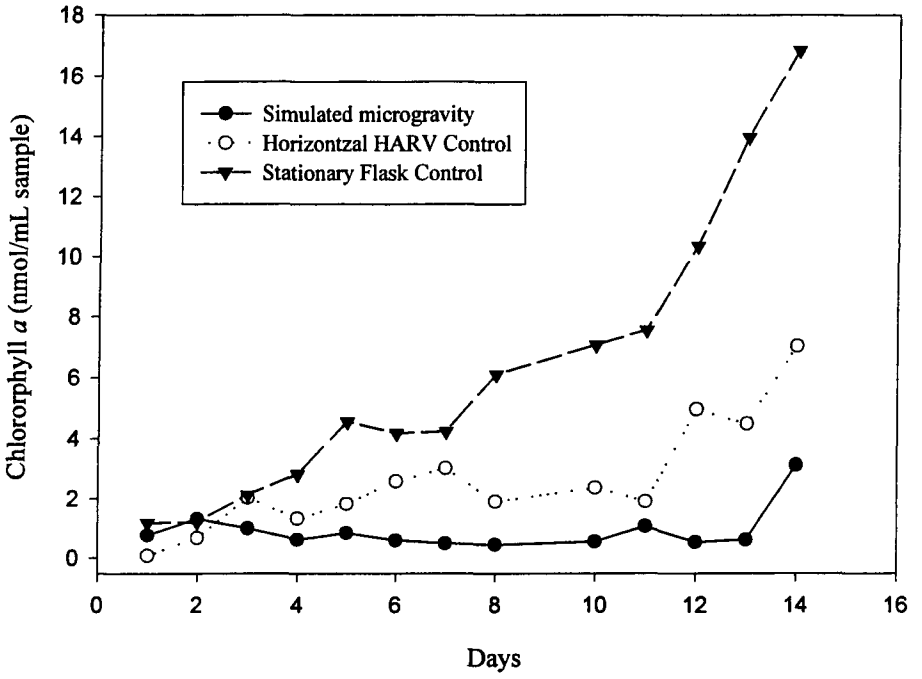

Figure 4. Effect of Simulated Microgravity on Chlorophyll *a* Production in *Chlorella pyrenoidosa* Cells

As noted above, *Chlorella* cells grew more slowly in the HARV under simulated microgravity than in the gravity control (Figure 2). These results are in contrast with the increase in growth, reproduction and viability of *Chlorella* cells seen in true microgravity when cultured on solid medium in space vehicles [9,10]. On the other hand, the decline in chlorophyll content of the *Chlorella* cells observed in simulated microgravity (Figure 4) is consistent with a corresponding decline in chlorophyll content seen when *Chlorella* cells were grown for 10.5 or 18 days in space on semiliquid medium.

CO_2 photoassimilation is often used as an index of the fitness of photosynthetic organisms. Table 2 illustrates the effect of simulated microgravity on CO_2-dependent O_2 evolution, which is a measure of CO_2 fixation capacity. The data are represented as O_2 evolution per cell or per unit chlorophyll. With either index, O_2 evolution rates were relatively similar for all samples. Thus, even though the growth rate was lower for cells cultured in simulated microgravity, the photosynthetic capacity of the cells appears to be unaffected.

Table 2. Effect of Simulated Microgravity on Photosynthetic Activity in *Chlorella pyrenoidosa* Cells as Determined by CO_2-Dependent O_2 Evolution.

Treatment	Photosynthetic activity (μmol O_2 evolved/min/ mg chlorophyll *a*)	Activity (% of stationary flask control)	Photosynthetic activity (μmol O_2 evolved /min/10^7 cells)	Activity (% of stationary flask control)
Stationary flask control	5.51	100	17.5	100.0
Horizontal HARV control	5.79	105.1	14.0	80.0
Simulated microgravity	5.47	99.3	14.1	80.9

An additional interesting finding is that cells grown in simulated microgravity form loose clumps or aggregates within 2 days of culture (Figure 5 and 6), with aggregation increasing with time of cell growth (Figures 7-12). A limited amount of cell clumping is also seen in cultures grown in the gravity control; however, the aggregation occurs later, for example after 7-14 days of growth, and only to a limited extent (Figures 7-12). At present, the basis for, or significance of, the cell aggregation is unknown.

Due to time limitations, proposed protein and gene expression studies were not completed. However, cell samples grown for 7 or 14 days under control and simulated microgravity conditions have been collected and stored in the ultracold; they will be used for subsequent experiments on protein and gene expression.

Conclusions

The initial results from this study suggest that cell growth and morphological characteristics of green algae may be affected by culture in simulated microgravity. The results obtained thus far should provide a solid basis for additional experiments regarding the effects of simulated microgravity on cell morphology, physiological activity, protein production and possibly gene expression in algal and plant systems. The final aim of the study is to provide useful information in elucidating the underlying mechanisms for the biological effects of microgravity on plant cells.

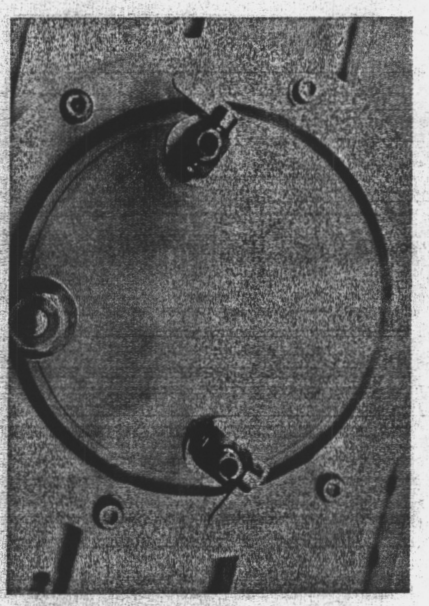

Figure 5. Appearance of *Chlorella* Cell Cultures Grown for 2 Days in a HARV Bioreactor Under Gravity Control Conditions.

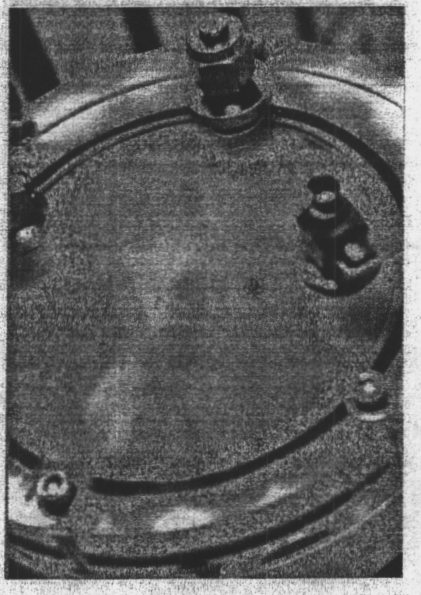

Figure 6. Appearance of *Chlorella* Cell Cultures Grown for 2 Days in a HARV Bioreactor Under Simulated Microgravity Conditions.

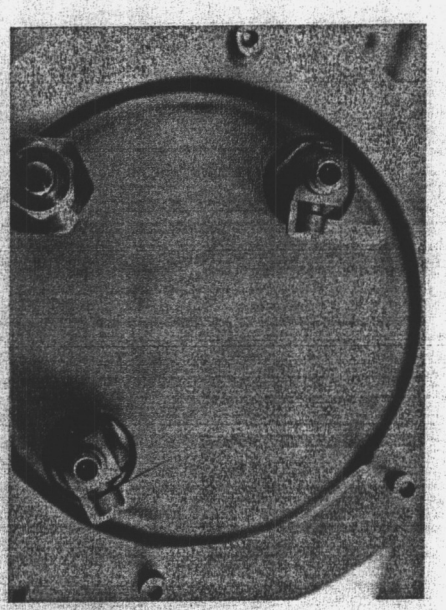

Figure 7. Appearance of *Chlorella* Cell Cultures Grown for 7 Days in a HARV Bioreactor Under Gravity Control Conditions.

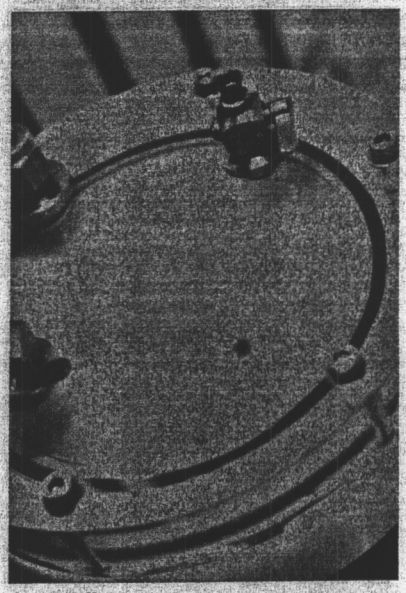

Figure 8. Appearance of *Chlorella* Cell Cultures Grown for 7 Days in a HARV Bioreactor Under Simulated Microgravity Conditions.

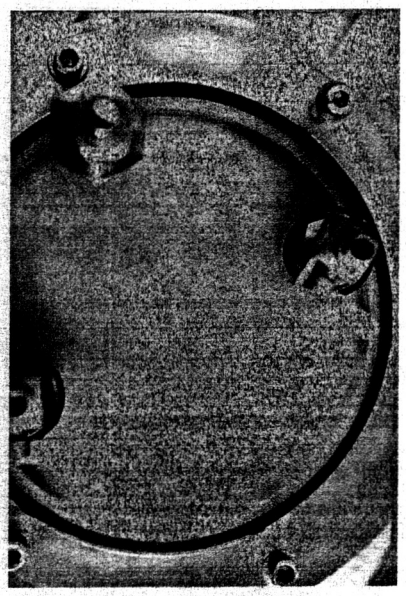

Figure 9. Appearance of *Chlorella* Cells Grown for 14 days in HARV Bioreactor Under Gravity Control Conditions.

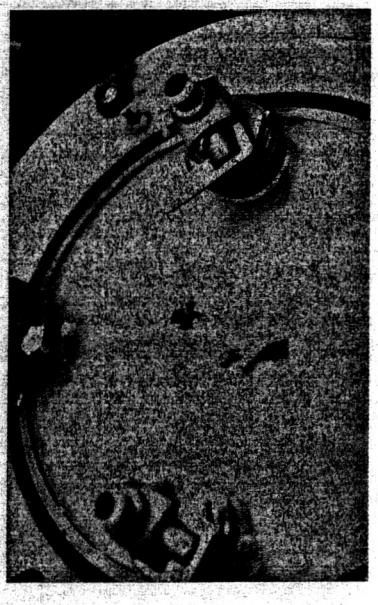

Figure 10. Appearance of *Chlorella* Cell Cultures Grown for 14 Days in HARV Bioreactor Under Simulated Microgravity Conditions.

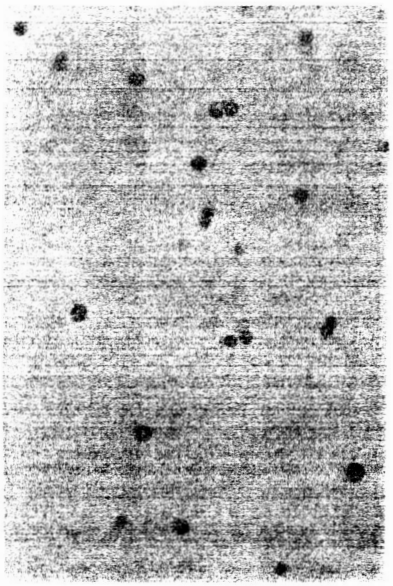

Figure 11. Microscopic Appearance of *Chlorella* Cells Grown for 14 Days in a HARV Bioreactor Under Gravity Control Conditions.

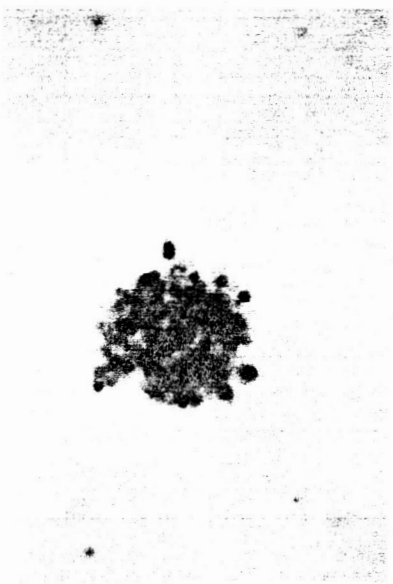

Figure 12. Microscopic Appearance of *Chlorella* Cells Grown for 14 Days in a HARV Bioreactor Under Simulated Microgravity Conditions.

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